Simple Method for the Isolation of Riboflavin from Whey

Abstract

A convenient, facile method for the isolation of riboflavin from whey is described. Whey is passed over a column of the neutral resin Amberlite XAD-2, on which riboflavin is adsorbed. Recoveries of the riboflavin with acetone as the eluant were satisfactory. The method is also convenient for removing riboflavin when it interferes in the analysis of other constituents.

Introduction

Although riboflavin is now readily available in synthesized form, it is expensive. Therefore, the need for an inexpensive, facile way to isolate riboflavin from dairy products is of interest. Leviton (1) removed riboflavin by adsorption on crystallized lactose from whey concentrates. Koziolowa (2) successfully used ion exchange resins for the isolation of flavins in certain food products. We noticed that when milk was passed over a column of neutral resin Amberlite XAD-2, a yellowish-green band formed at the top of the column. This observation led us to investigate the feasibility of using this resin for the extraction and recovery of riboflavin from whey.

Experimental Procedure

A standard solution of riboflavin[1] was prepared by dissolving 10 mg in 250 ml of redistilled H_2O. A column of the resin Amberlite XAD-2[2] was prepared by slurrying 4 g of the resin in redistilled water and pouring into a glass chromatographic column (1.3 cm id × 30 cm). The column was then washed with 5 to 10 column volumes of water, 5 column volumes of redistilled methanol,[3] and again with 5 column volumes of redistilled water. The column was maintained in water.

Recoveries were made of the pure riboflavin by diluting 1, 2, 3, and 4 ml of the standard solution to 10 ml with redistilled water. The individual solutions were passed over the column at about 4 ml/min. Recoveries were made by adding 1, 2, 3, and 4 ml of the standard solution to 50 ml of sweet cheese whey and passing the solution over the column at 4 ml/min. After application of the solutions to the resin, the column was washed with 5 column volumes of redistilled water or until the eluate was clear. The adsorbed riboflavin was then eluted with 30 ml of redistilled acetone.[3] The acetone eluate was evaporated on a steam bath under a stream of nitrogen, and the residues were dissolved in 10 ml of water. The amount of riboflavin was then determined spectrophotometrically at wavelength 447 nm (3)

[1] Matheson, Coleman, and Bell, East Rutherford, New Jersey.

[2] Rohm and Haas, Philadelphia, Pennsylvania.

[3] Baker Analyzed, Baker Chemical Company, Phillipsburg, New Jersey.

Results and Discussion

The results in Table 1 indicate satisfactory recoveries of riboflavin by adsorption and desorption from the resin. The loss of about 10% can probably be attributed to the irreversible adsorption of riboflavin by the column or deactivation of riboflavin by light. Acetone is the best solvent for removing adsorbed riboflavin. The column must be moist when the acetone is added; if not, poorer extractions are realized.

TABLE 1. Recovery of riboflavin.

Dilutions	Reading (447 nm)	Recovered duplicates		Recovered
				(%)
1:10	.125	.115	.115	91
2:10	.255	.225	.220	88
3:10	.375	.335	.330	89
4:10	.500	.45	.46	91

However, acetone-water mixtures did not increase yields. Whereas hot water was an effective eluant, methanol and ethanol were almost as efficient as acetone.

Table 2 shows that when riboflavin was added to the whey, the recoveries were lower but

TABLE 2. Recoveries using whey plus riboflavin.

Riboflavin added to whey	Recovered duplicates		Recovered
(ml)			(%)
0	.155	.160	
1	.240	.235	85
2	.340	.335	83
3	.44	.44	83
4	.55	.56	84

satisfactory. The loss is probably due to binding of added riboflavin by whey protein, thus preventing the riboflavin from being adsorbed.

The method conveniently isolates natural, pure riboflavin. Because the resin is relatively inert and neutral, resin-induced transformations (2) are minimized. The capacity of the column was about 1 mg for a 4-g column at the designated flow rate. This could be increased by reducing the flow rate.

The method is economically advantageous. It can be scaled up thereby utilizing whey as a source of riboflavin. The materials are relatively inexpensive and can be recovered and used repeatedly.

Not only is the method suitable for the recovery of riboflavin, but it conveniently removes riboflavin when it interferes with analyses of other milk constituents. In addition, it would be useful in other areas, e.g., protein extracts, where riboflavin and other flavins interfere with analyses.

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References

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